Translational Genomics: A Case Study of the Rice *Pi-ta* Resistance Gene *Pi-ta*

Jia Yulin[1*] Zhou Erxun[1, 2, 3] Winston Eugenia[1] Singh Pratibha[4] Wang Zhonghua[5] Correll James.[6] Lee Fleet.[4] Jia Melissa[1]

1 US Department of Agriculture-Agriculture Research Service, Dale Bumpers National Rice, Research Center, Stuttgart, AR 72160, USA; 2 Rice Research and Extension Center, University of Arkansas, Stuttgart, AR 72160, USA; 3 South China Agricultural University, Guangzhou 510642, P. R. China; 4 Cornell University, Ithaca, NY 14850, USA; 5 Zhejiang Wanli University, Ningbo 315100, P.R. China; [6] University of Arkansas, Fayetteville, AR 72701,USA

* Corresponding author, yjia@spa.ars.usda.gov

* , yjia@spa.ars.usda.gov

ABSTRACT

Blast disease is one of the most destructive rice diseases worldwide. The *Pi-ta* resistance gene is one of those *R* genes characterized at the molecular level. *Pi-ta* encodes a putative cytoplasmic protein with nucleotide binding sites and a leucine rich repeat. *AVR-Pita* encodes a metalloprotease, and its processed form AVR-Pita $_{176}$ interacts directly with the *Pi-ta* protein to trigger complete resistance to the race of *Magnaporthe grisea* containing the corresponding avirulence gene *AVR-Pita*. One resistant *Pi-ta* and three susceptible *pi-ta* alleles were identified from rice germplasm. An alignment of DNA sequences of these *Pi-ta* haplotypes identified several conserved nucleotide substitutions, and these nucleotide differences were used to develop PCR based markers for stacking the *Pi-ta* gene into advanced rice breeding lines by marker-assisted selection (MAS). Similarly, results of structural and functional studies of *avr-pita* alleles in US *M. grisea* pathotypes suggests that transposition, insertion and deletion of DNA sequences at the *AVR-Pita* locus may be responsible for the instability of *AVR-Pita*, and thus it is a likely mechanism to defeat the resistance mediated by *Pi-ta*. A combination of genetic and biochemical approaches has been undertaken to understand the coevolution of a plant *R* gene and a corresponding pathogen *AVR* gene. Recent progress in developing the molecular methods for rice breeding and crop protection will be presented.

Keywords: disease resistance, molecular marker assisted breeding, rice genomics, blast disease, Pi-ta

INTRODUCTION

Evolutionary studies clearly indicate that plants are coevolving with pathogens. Plants have developed a multifaceted and sophisticated defense system to fight pathogens, and the pathogens have evolved dynamic strategies to defeat the plant defense. Knowledge of this never-ending arms race between plants and pathogens can benefit crop protection. Clearly, an effective defense response is often mediated by major plant resistance (R) genes to fight biotrophic or hemibiotrophic pathogens where the live cells are essential for invasive growth, such as *Magnaporthe grisea*, the casual agent of rice blast disease. Rice blast continues to be a major threat to rice production worldwide (Lee, 1994; Jia, 2003). The mechanisms of interactions of hosts and pathogens have been investigated at the molecular level. Resistance to biotrophic and

hemibiotrophic pathogens is often governed by a gene-for-gene relationship. A resistance (*R*) gene is effective in preventing infection by the pathogen containing the corresponding avirulence (*AVR*) gene. Molecular characterization of over 48 plant *R* genes has advanced the ability to breed for effective resistance against pathogens. Knowledge of DNA sequences of susceptible and resistant alleles not only enhances our understanding of *R* gene evolution, but can also be used to identify nucleotides that distinguish the resistant counterpart. These unique DNA sequences can be used to develop *R* gene specific primers to amplify portions of an *R* gene as a marker for marker-assisted selection (MAS). Even though a number of *R* genes for blast resistance have been identified in rice cultivars (Yu et al., 1996), the durability of resistance mediated by a single *R* gene is still inadequate for disease control due to rapid development of virulent races in the field (Valent, 1997). In this paper, a case study of a rice blast resistance gene *Pi-ta* is presented.

1 THE *PI-TA* GENE

The *Pi-ta* gene in rice is effective in preventing the infection of *Magnaporthe grisea* isolates containing the corresponding avirulence gene *AVR-Pita* in a gene-for-gene fashion (Flor, 1971; Jia et al., 2000). The *Pi-ta* gene is mapped near the centromere of chromosome 12, a relatively stable region due to suppressed recombination at the centromere region (Chen et al., 2002). From 1995 to 2000, positional cloning of the *Pi-ta* gene was intensively pursued in Japan and US (Rybka et al., 1997; Bryan et al., 2000). *Pi-ta* is a single copy gene and it is constitutively expressed in low abundance and obvious induction of the *Pi-ta* gene has not been observed. *Pi-ta* is predicted to encode an interrupted open reading frame with an intron.

The Pi-ta protein is predicted to be a cytoplasmic protein with 928 amino acids with a centrally localized nucleotide-binding site and an imperfect leucine rich repeat (leucine rich domain, LRD) at the carboxyl terminus. Remarkably, the data from transient expression, the yeast two-hybrid system, and *in vitro* binding suggest AVR-Pita is processed to AVR-Pita$_{176}$, the putative elicitor that triggers the *Pi-ta* dependent defense response (Jia et al., 2000). Pi-ta is a putative receptor protein and the LRD at its carboxyl terminus might be involved in binding to the elicitor (Bryan et al., 2000; Jia et al., 2000). The *AVR-Pita* gene is thus proposed to be an elicitor to bind the Pi-ta protein, a putative cytoplasmic receptor, to trigger an effective defense response-elicitor/receptor model (Jia et al., 2000). Physical interaction of Pi-ta with AVR-Pita provides indirect evidence that the AVR-Pita protein is processed and secreted into the plant cell. It is possible that hemibiotrophic and biotrophic fungi employ specialized secretion systems for delivering proteins into the cytoplasm of their host cell without killing the cell. AVR-Pita is a putative metalloprotease that has been proposed to be able to process the Pi-ta protein. It is still unknown whether or not AVR-Pita cleaves other fungal and plant proteins. Investigating *AVR-Pita* interacting genes certainly will shed light on whether AVR-Pita plays a role in degrading Pi-ta during recognition. The recombinant AVR-Pita$_{176}$ protein was expressed in a cDNA library in the yeast two-hybrid system. One of the AVR-Pita interacting proteins, AVR3, is a putative membrane associated protein. AVR3 is a single copy gene. The fungal elicitor induced the expression of the AVR3 gene. Functional confirmation of the *AVR3* gene is in progress.

Regardless of how the Pi-ta protein functions in detecting the AVR-Pita protein, one of the significant outcomes of *R* gene-mediated resistance is to activate defense responses. Under heavy artificial inoculations, rapid transcript accumulation of defense genes PR-1, ß-glucanase and chitinase within 24 hrs in plants containing the *Pi-ta* gene suggests that plants responded to the pathogen infection quickly and these defense genes could be used as RNA markers to detect early resistance response to blast. To establish a link between signal recognition and transduction, a genetic screening was undertaken to identify blast susceptible mutants of Katy, a cultivar containing the *Pi-ta* gene (Jia et al., 2005). Thus far, three fast neutron induced blast susceptible mutants containing the *Pi-ta* gene are being crossed to Katy to identify novel loci that interact with the *Pi-ta* gene. The loss of function of the *Pi-ta* gene was also identified in two fast neutron induced blast susceptible

mutants and precise determination of the DNA sequence of the *Pi-ta* gene will provide more information about critical regions for Pi-ta function. More straightforward, the benefit of cloning an *R* gene is to incorporate the cloned *R* gene into diverse advanced breeding lines for improved blast resistance by transformation. However, this approach is currently restricted by the intellectual property issue and customer perception of a genetically modified organism.

An additional benefit of cloning an *R* gene is to identify sequence variation of its susceptible counterpart, such information is useful for identifying critical amino acids and domains for its resistance function. Analysis of rice cultivars from major rice growing areas including China, Colombia, Japan, Philippines identified four *Pi-ta* haplotypes (Jia et al., 2003). Rice cultivars containing one resistant *Pi-ta* haplotype and three susceptible *pi-ta* haplotypes were discovered. Orthologous comparisons identified five amino acid differences between the indica *Pi-ta* allele and the japonica *pi-ta* alleles. Different nucleotides were identified from different *Pi-ta* haplotypes at identical positions on the *Pi-ta* gene. Invariably, a single amino acid was identified distinguishing resistance with alanine at position 918 and susceptibility when alanine is replaced with serine at position 918. A single nucleotide difference with alteration of alanine to serine is called a functional nucleotide polymorphism (FNP). The occurrence of FNP in the Pi-ta protein is a favorable explanation of how remarkable it is that plants have evolved to detect rapid evolution of pathogen signal molecules.

Highly conserved nucleotide sequences in rice accessions enabled us to develop perfect DNA markers to tag the *Pi-ta* gene. These markers are dominant or codominant, and are suitable for both low cost agarose gel based analysis and automation assisted marker-assisted breeding respectively (Jia et al., 2002; Jia et al., 2004a and b; Johnson et al., 2005). In the US, a Vietnam indica landrace cultivar Tetep was *Pi-ta* donor for an elite tropical japonica, Katy, in 1990 (Moldenhauer et al., 1990). Subsequently Katy was used as the donor for *Pi-ta* in the elite US cultivars Madison, Drew, Kaybonnet, Cybonnet, and Ahrent. In Japan, a Philippine landrace indica cultivar Taduca was *Pi-ta* donor for elite cultivars Pi-1, Pi-2, Pi-3, Pi-4, Pi-5, Reiho, Shimokita and Yashiro-mochi. Using these powerful markers, the resistant *Pi-ta* alleles were identified from the Chinese indica cultivars, Teqing and Guichao 2. Both Teqing and Guichao2 are resistant to *M. grisea* isolates containing *AVR-Pita*.

Evidence that *Pi-ta* occurs spontaneously from landrace varieties is accumulating. It is possible that *Pi-ta* variants in red rice, a competitor of rice may have been evolved to be *R* genes for resistance to *M.grisea* races in the areas where *Pi-ta* containing cultivars are cultivated (Correll et al., 2004, Lee et al., 2005). Several ecotypes of red rice also were shown to be resistant to both IE-1K and IB-33 (F.N. Lee and D. Gealy, personal. comm.). We are now correlating the structural variation of *Pi-ta* alleles in these ecotypes with their disease reactions.

Low stringy Southern blot analysis indicates that a homologous DNA sequence of the *Pi-ta* gene also exists in other cereals including barley, maize, oat, rye, and wheat, and also crabgrass, foxtail millet, and weeping lovegrass. However, orthologs of the *Pi-ta* gene are only found in maize and rye. Structural and functional characterization of these *Pi-ta* orthologs will help to understand whether or not they are also *R* genes.

2 *M. grisea AVR-Pita* GENE

The *M. grisea AVR-Pita* gene determines efficacy for the *Pi-ta* gene and is located at a telomeric region of chromosome 3 (Orbach et al., 2000). *AVR-Pita* encodes a metalloprotease where a conserved zinc protease motif was identified to be important for its function (Jia et al., 2000). The *AVR-Pita* gene in prevalent US *M. grisea* isolates as well as isolates collected 30 years ago were investigated recently. A total of 154 isolates were determined to contain a functional homolog of the *AVR-Pita* gene (Winston et al., 2005). Preliminary sequence analysis identified minor changes in the *AVR-Pita* genes among different isolates/races. Uniformly, *M. grisea* isolates infecting rice cultivars containing the *Pi-ta* genes had lost the function of the *AVR-Pita* gene. Evidence indicating *M, grisea* transposon Pot3 insertion, amino acid insertion and deletion

of the *AVR-Pita* gene has been accumulated from commercial rice fields and laboratories (Kang et al., 2001; Zhou et al., 2005). Progress in developing allele specific primers to monitor the *AVR-Pita* genes for predicting the stability of *Pi-ta* resistance is being made. Deletions of *AVR-Pita* are most interesting because *AVR-Pita* is located in the telomere repeat of an *M. grisea* chromosome (Orbach et al., 2000).

3 DURABLE BLAST RESISTANCE

Pi-ta has been used effectively to control rice blast disease in the Southern US for several decades. Durability of *Pi-ta* in the southern US occur because 1) conditions for epidemics have been marginal and/or 2) virulent races that lack *AVR-Pita* may not be fit for southern rice growing areas. Similarly, the deployment of rice cultivars with diverse genetic backgrounds in integrated cultural practices also likely accounts for the effectiveness of the blast resistance provided by *Pi-ta*. Research has progressed toward understanding the role of *AVR-Pita* in fitness. Resulting knowledge not only will provide insights into molecular mechanisms of evolution of the *M. grisea* avirulence gene, but also will be useful in predicting the stability of blast resistance in rice cultivars that are currently grown in the southern US. The occurrence of virulent strains of *M. grisea* demands additional blast resistance genes for achieving a durable blast resistance. Over the years, *M. grisea* virulent races IE-1K and IB-33 that have overcome the resistance provided by *Pi-ta* have been recovered from both the laboratory and field (Lee et al., 2005). Rice cultivars Raminad Strain 3 and Zhe733, resistant to both IE1K and IB-33 (Wamishe et al., 2005), have been chosen for introducing new resistance into US breeding lines.

4 SUMMARY

The *M. grisea* is a cereal killer. Its resulting rice blast is a major challenge in rice production worldwide, particularly for upland rice where rice is dependent on rain fed irrigation. Integrated cultural practices, including the use of fungicides, have effectively protected rice crops, however, the continued and increased using of fungicides will ultimately have environmental consequences. Therefore, an improved understanding of the function of the *R* and *AVR* genes in the rice pathosystem will ultimately help us to better manage this important disease. The structural and functional characteristics of the *Pi-ta* gene are now better understood. This new knowledge has provided valuable insights into the molecular mechanisms of R gene mediated defense response and also has accelerated the development of improved resistance to blast. The availability of the complete sequence of the rice genome (Burr et al., 2005), high-density genetic and physical maps (McCouch et al., 1998; Chen et al., 2002), large expression profiles, and amenable transformation systems are certainly important tools for translational genomics.

ACKNOWLEDGEMENT

We thank Michael Lin, Lin Wu, Meiyan Zhou, CuWanda Flowers, Andrea Blas for excellent technical support. This project was supported in part by University of Arkansas Rice Research and Promotion Board and Zhejiang University for Z. Wang.

REFERENCES

Bryan B.T., Wu K.S., Farrall L., Jia Y., Hershey H., McAdams S., Tarchini R., Donaldson G., Faulk K., and Valent B., 2000, A single amino acid difference distinguishes resistant and susceptible allele of the rice blast resistance gene *Pi-ta*, Plant Cell, 12: 2033-2045

Burr B., 2005, The map-based sequence of the rice genome, Nature, 436: 793-800

Chen M., Presting G., Barbazuk W.B., 2002, An integrated physical and genetic map of the rice genome, Plant Cell, 14: 537-545

Correll J.C., Jia Y., Boza E.J., Singh P., and Lee F.N., 2005, How does the rice blast pathogen defeat a resistance gene, Proced. of the 30th Rice Technical Working Group Meeting, Feb. 29-March 3, 2004, New Orleans, LA, pp104

Flor H.H., 1971, Current status of the gene-for-gene concept, Annu. Rev. Phytopathol., 9: 275-296

Jia Y., 2003, Marker assisted selection for the control of rice blast disease, Pesticide Outlook, 14: 150-152

Jia Y., Bryan G.T., Farrall L., and Valent B., 2003, Natural variation at the *Pi-ta* rice blast resistance locus, Phytopathology, 93: 1452-1459

Jia Y., Lin M.J., and Jia M.H., 2005, Genetic analysis of the rice blast resistance gene *Pi-ta*-mediated signal transduction pathway, Phytopathology, 2005, APS, Austin, Texas, Phytopathology Phytopathology, 95: S48

Jia Y., McAdams S.A., Bryan G.T., Hershey H.P., and Valent B., 2000, Direct interaction of resistance gene and avirulence gene products confers rice blast resistance, EMBO J., 19: 4004-4014

Jia Y., Redus M., Wang Z., and Rutger J. N., 2004a, Development of a SNLP marker from the *Pi-ta* blast resistance gene by tri-primer PCR, Euphytica, 138: 97-105

Jia Y., Wang Z., and Singh P., 2002, Development of dominant rice blast resistance *Pi-ta* gene markers, Crop Sci., 42: 2145-2149

Jia Y., Wang Z., Fjellstrom R.G., Moldenhauer K.A.K., Azam M.A., Correll J., Lee F.N., Xia Y., and Rutger J.N., 2004b, Rice *Pi-ta* gene confers resistance to the major pathotypes of the rice blast fungus in the US, Phytopathology, 94: 296-301

Johnson V.A., Redus M.A., Gibbons J.W., Moldenhauer K.A.K., Jiang J., and Jia Y., 2005, Marker-assisted selection for the rice blast resistance gene *Pi-ta*: Development and use of an improved codomiant analysis method, Proced. of the 30th Rice Technical Working Group Meeting, Feb. 29-March 3, 2004, New Orleans, LA. pp.62

Kang S., Lebrun M.H., Farrall L., and Valent B., 2001, Gain of virulence caused by insertion of a Pot3 transposon in a *Magnaporthe grisea* avirulence gene, Mol. Plant Micro. Interact., 14: 671-674

Lee F.N., 1994, Rice breeding programs, blast epidemics and blast management in the United States, In: Zeigler R.S., Leong S.A., and Teng P.S. (eds.), Rice blast disease, CAB Int, Wallingford, UK, pp.489-500

Lee F.N.,.Cartwright R.D, Jia Y., Correll J.C.,. Moldenhauer K.A.K, Gibbons J.W., Blytt V., Zhou E., Boza E., and Seyran E., 2005, A preliminary characterization of the rice blast fungus on 'Banks" rice, In: Wells B.R., Norman R.J., and Meullenet J.F. (eds.), 2004, Rice Research Studies, Arkansas Agricultural Experiment Station, series (accepted)

McCouch S.R, Kochert G., Yu Z., Wang Z., Khush G.S., Coffman W.R., and Tanksley S.D., 1988, Molecular mapping of rice chromosomes, Theor. Appl. Genet., 76: 815-829

Moldenhauer K.A.K., Lee F.N., Norman R.J., Helms R.S., Well R.H., Dilday R.H., Rohman P.C., and Marchetti M.A.. 1990, Registration of 'Katy' rice, Crop Sci., 30: 747-748

Orbach M.J., Farrall L., Sweigard J., Chumley F.G., and Valent B., 2000, A telomeric avirulence gene determines efficacy for the rice blast resistance gene *Pi-ta*, Plant Cell, 12: 2019-2032

Rybka K., Miyamoto M., Ando I., and Kawasaki S., 1997, High resolution mapping of the indica-derived rice blast resistance genes, II. *Pi-ta2* and *Pi-ta* and a consideration of their origin, Mol. Plant-Microbe Interact., 10: 517-524

Valent B., 1997, The rice blast fungus, *Magnaporthe grisea*, In: Carroll/Tudzynoski (eds.), Plant relationships, The Mycota V Part B., Springer-Verlag, Berlin Heidelberg, pp.37-54

Wamishe Y.A., Jia M., and Jia Y, 2005, Identification of blast resistance genes in rice cultivar Zhe733, Phytopathology, 2005 APS, Austin, Texas, Phytopathology, 95: S108

Winston E., Jia Y., and Correll J., 2005, Molecular Characterization of *Magnaporthe grisea* Avirulence Alleles (*AVR-Pita*) in US Pathotypes, Proced. of the 30th Rice Technical Working Group Meeting, Feb 29-March 3, 2004, New Orleans, LA, pp.110

Yu Z.H., Mackill D.J., Bonman J.M., McCouch S.R., Guiderdoni E., Notteghem J.L., and Tanksley S.D., 1996, Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea* Sacc.), Theor. Appl. Genet., 93: 859-863

Zhou E., Jia Y., Lee F.N., Lin M.J., Jia M.H., Correll J.C. and Cartwright R., 2005, Evidence of the instability of a telomeric *Magnaporthe grisea* avirulence gene *AVR-Pita* in the U.S., Phytopathology, 2005 APS, Austin, Texas, Phytopathology 95: S118